

Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1. (Currently amended) An isolated polynucleotide molecule comprising an ARS nucleotide sequence, having the function of enhancing the transformation efficiency and the maintenance of vectors as stable extrachromosomal elements in yeast, said ARS nucleotide sequence having at least 95% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 2 and SEQ ID NO. 3.

2. (Original) The isolated polynucleotide molecule of claim 1 wherein said polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 2 and SEQ ID NO. 3.

3. (Original) A vector comprising the isolated polynucleotide molecule of claim 1.

4. (Original) A vector comprising the isolated polynucleotide molecule of claim 2.

5. (Original) The vector of claim 4, wherein said vector is selected from the group consisting of pCfARS6, pCfARS11, pCfARS16, pCfARS1614, pCfARS68,

PRpL2, pPgARS11, pPgARS12, pPgARS13, pPgARS14, pPgARS15, pPgARS16, pPgARS17, pRIV-1, pRIV-2, pCR7, PRpRIV-1, pCR1, PRp1, pCR1Xb, pCR2, PRp2, PRp1Xb, pCR2-1, pCR2-21, pCR2-22, PRp2-11, PRp2-12, PgARS19, PRpIV-2, p19R7RIV-2, p19R7RIV-22, pD, pG, pE, pB, pF, pC, pA, pPR5, pCPR5, pPR5, pFC, pFCL-2, PRp7, PRSp1Xb, PRSp2-11, pCPSR5, pFCLS2, PRSp7, pRIB7, p2R, pR15, pR157, pR1572, pR2 and pRIB1.

6. (Original) Isolated or purified cells comprising the vector of claim 3.
7. (Original) Isolated or purified cells comprising the vector of claim 4.
8. (Original) The cells of claim 7 wherein said cells are yeast cells.
9. (Original) The yeast cells of claim 8, wherein said yeast is a flavinogenic yeast.
10. (Original) The yeast cells of claim 9 wherein said yeast cells are *Candida* or *Pichia*.
11. (Original) The yeast cells of claim 10 wherein said yeast cells are *Candida famata* VKM Y-9 L20105 having NRRL deposit number Y-30292.

12. (Original) The yeast cells of claim 7, wherein said yeast cells comprise a gene library selected from the group consisting of: a gene library comprising vectors comprising *Pichia guilliermondii* ATCC 9058 DNA segments, PgARS elements, and CfARS elements; and a gene library comprising vectors comprising *Candida famata* VKM Y-9 DNA segments, CfARS elements and PgARS elements.

13. (Original) A method for the transformation of yeast cells comprising electroporating a cell suspension containing said yeast together with one or more nucleic acid constructs comprising one or more regulatory sequences and one or more genes or gene segments using one or more of resistance, field strength and pulse duration sufficient to transform said cells, wherein said constructs comprise a polynucleotide molecule of claim 1.

14. (Original) The method of claim 13 where said construct comprises a nucleotide sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 2 and SEQ ID NO. 3.

15. (Original) A method for the transformation of yeast cells comprising electroporating a cell suspension containing said yeast together with one or more nucleic acid constructs comprising one or more regulatory sequences and one or more genes or gene segments using one or more of resistance, field strength and pulse duration sufficient to transform said cells, wherein said field strength is from about 8 to about 15 kV/cm.

16. (Original) The method of claim 15, wherein said resistance is from about 13 ohms to about 720 ohms.

17. (Original) The method of claim 16, wherein said resistance is about 129 ohms.

18. (Original) The method of claim 15, wherein said pulse duration is from about 1 ms to about 10 ms.

19. (Currently amended) A method for the transformation of yeast cells comprising electroporating a cell suspension containing said yeast together with one or more nucleic acid constructs comprising one or more regulatory sequences and one or more genes or gene segments using one or more of resistance, field strength and pulse duration sufficient to transform said cells, wherein said cell suspension comprises 0.3 M to 1.0 M sucrose.

20. (Original) A method for the transformation of yeast cells comprising providing spheroplasts of said yeast cells, contacting a solution comprising said spheroplasts with one or more nucleic acid constructs comprising one or more regulatory sequences and one or more genes or gene segments and with one or more fusion agents, for a time sufficient to transform said spheroplasts, wherein said constructs comprise a polynucleotide molecule of claim 1.

21. (Original) The method of claim 20 where said construct comprises a nucleotide sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 2 and SEQ ID NO. 3.

22. (Withdrawn) Isolated or purified *Candida famata* cells selected from the group consisting of:

- (1) Leaky *rib2* mutants of *Candida famata* VKM Y-9 (*leu2⁻rib2⁻*) wherein said mutants grow without exogenous riboflavin and in the absence of cobalt addition;
- (2) *Candida famata ts rib1* mutants which overproduce riboflavin in both iron-sufficient and iron-deficient media;
- (3) *Candida famata* mutants of part (b) which overproduce riboflavin at reduced temperature;
- (4) *Candida famata ts rib1* mutants which overproduce riboflavin in iron-sufficient media;
- (5) *Candida famata ts rib1* mutants which overproduce riboflavin in iron-deficient media;
- (6) *Candida famata* leaky *rib2* mutants, wherein said mutants grow without exogenous riboflavin and in the presence of Co^{+2} ions;
- (7) *Candida famata ts rib1* mutants which are protrophic for riboflavin production at reduced temperatures and which retain riboflavin auxotrophy at elevated temperatures;

- (8) *Candida famata* ts rib5/rib6 mutants which are prototrophic for riboflavin production at reduced temperatures and which retain riboflavin auxotrophy at elevated temperatures;
- (9) *Candida famata* leaky rib5/rib6 mutants, wherein said mutants grow without exogenous riboflavin in the presence of exogenous Co⁺² ions and which are riboflavin auxotrophic in medium without exogenous Co⁺² ions;
- (10) *Candida famata* ts rib5/rib6 mutants which produce riboflavin in iron sufficient media and which grow in the presence of Cu⁺² ions;
- (11) Leaky rib2 mutants of *Candida famata* VKM Y-9 (*leu2⁻rib2⁻*) wherein said mutant comprises sufficient rib2 gene encoded enzyme (reductase) activity to retain riboflavin auxotrophy under conditions of flavinogenesis enzyme repression and simultaneously sufficient reductase activity to produce riboflavin prototrophy under conditions of flavinogenesis enzyme derepression;
- (12) The leaky mutants of (k) wherein said condition of flavinogenesis enzyme repression is growth in iron sufficient media;
- (13) The leaky mutants of (k) wherein said condition of flavinogenesis enzyme derepression is growth in iron deficient media;
- (14) *Candida famata* mutant #105 1-2 (*leu2*), wherein said mutant grows on ethanol as the sole carbon and energy source and which produces about 70-100 µg riboflavin/ml, having NRRL deposit no. Y-30455;
- (15) *Candida famata* VKM Y-9 L20105 having NRRL deposit no. Y-30292;

(16) *Candida famata* VKM Y-9 (*leu2⁻rib⁻*) mutants selected from the group consisting of:

- 1) mutants having a genetic block of the *rib1* gene;
- 2) mutants of (1) transformed with a vector selected from the group consisting of PRp1, pCR1Xb, PRp1Xb and pCR1; wherein said vector comprises a nucleic acid segment which provides complementation of said genetic block when said nucleic acid segment is expressed in said mutant;
- 3) mutants having a genetic block of the *rib2* gene;
- 4) mutants of (3) transformed with a vector selected from the group consisting of pCR2, pCR2-1, and pR2; wherein said vector comprises a nucleic acid segment which provides complementation of said genetic block when said nucleic acid segment is expressed in said mutant;
- 5) mutants having a genetic block of the *rib3* gene;
- 6) mutants having a genetic block of the *rib5* gene;
- 7) mutants of (6) transformed with a vector selected from the group consisting of pPR5, pRIV-2, and PRpIV-2; wherein said vector comprises a nucleic acid segment which provides complementation of said genetic block when said nucleic acid segment is expressed in said mutant;
- 8) mutants having a genetic block of the *rib6* gene;
- 9) mutants of (8) transformed with vector pF; wherein said vector comprises a nucleic acid segment which provides complementation of said genetic block when said nucleic acid segment is expressed in said mutant;

10) mutants having a genetic block of the *rib7* gene;

and

11) mutants of (10) transformed with a vector selected from the group consisting of pCR7, pPRp7 and pRIB7; and, wherein said vector comprises a nucleic acid segment which provides complementation of said genetic block when said nucleic acid segment is expressed in said mutant.

23. (Withdrawn) A method comprising:

(1) growing a transformed yeast under conditions that provide for synthesis of riboflavin,

wherein said transformed yeast comprises one or more nucleic acid constructs comprising one or more copies of one or more genes encoding one or more enzymes involved in riboflavin biosynthesis;

wherein said one or more nucleic acid constructs further comprise a polynucleotide molecule of claim 2;

wherein said riboflavin is synthesized by said transformed yeast, said synthesis being greater than that of the corresponding non-transformed yeast;

(2) recovering said riboflavin from said culture media in which said transformed yeast was cultured.

24. (Withdrawn) A method comprising:

(1) growing a mutant yeast strain under conditions that provide for synthesis of riboflavin,

wherein said mutant yeast strain produces riboflavin in both iron sufficient and iron deficient media;

wherein said riboflavin is synthesized by said mutant yeast strain, said synthesis being greater than that of the corresponding non-mutant parental yeast strain; and

(2) recovering said riboflavin from said culture media in which said mutant yeast was cultured.

25. (Withdrawn) The method of claim 24, wherein said mutant yeast strain is a *Candida famata ts rib1* revertant mutant and said parental strain is *Candida famata* VKM Y-9 (*leu2rib1*) L20105.

26. (Withdrawn) A method comprising:

(1) growing a mutant yeast strain under conditions that provide for synthesis of riboflavin,

wherein said mutant yeast strain produces riboflavin in iron sufficient media, and wherein ethanol is the sole energy and carbon source of said mutant;

wherein said riboflavin is synthesized by said mutant yeast strain, said synthesis being greater than that of the corresponding non-mutant parental yeast strain; and

(2) recovering said riboflavin from said culture media in which said mutant yeast was cultured.

27. (Withdrawn) A method comprising:

(a) culturing mutant yeast cells under conditions that provide for synthesis of riboflavin, wherein culturing comprises culture media comprising iron and chromium ions; wherein said mutant yeast cells are *Candida famata* VKM Y-9 L20105;

wherein said riboflavin synthesis is greater in said media comprising chromium and iron ions than when said mutant yeast is grown in media lacking chromium ions; and,

(2) recovering said riboflavin from said culture media in which said mutant yeast was cultured.

28. (Withdrawn) The method of claim 27 wherein said chromium is hexavalent chromium.

29. (Withdrawn) A method of obtaining flavinogenic yeast cells having one or more altered biological properties as compared to untreated cells comprising treating *Candida famata* NRRL 30292 cells at least once with one or more treatment agents under one or more treatment conditions for a time period sufficient to alter one or more biological properties, wherein one or more biological properties of said treated cells differs from biological properties of untreated cells.

30. (Withdrawn) The method of claim 29 wherein said treatment agents are

selected from the group consisting of hexavalent chromium, cadmium ions, cobalt ions, iron ions, copper ions, 2-monofluoroacetate, UV light, and nitrosoguanidine.

31. (Withdrawn) The method of claim 30 wherein said treatment conditions are selected from the group consisting of heat shock, cold shock, iron-deficient media, iron-rich media, and alcohol.